tives and also affects non-CNC derived cells as demonstrated by the accelerated recruitment of endothelial cells and osteoclasts. In contrast, mineralization of quail donor-derived osteoid occurs according to the duck host timetable, which suggests involvement of host-derived systemic factors. Thus, by using the quail–duck chimeric system, we have been able to distinguish those aspects of osteogenesis that are controlled locally by CNC-derived mesenchyme, and those aspects that are regulated systemically. Such data will be critical for identifying and targeting specific molecules as a means to manipulate the timing of bone formation and treat birth defects, traumatic injuries, and diseases that affect the skeleton.

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The TGF-β ligand derrière cooperates with Xnr1 and the nodal inhibitor Coco to establish the left–right axis in *Xenopus*

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In *Xenopus*, the nodal-like gene Xnr1 and its inhibitor Coco are coexpressed in the posterior paraxial mesoderm (node equivalent, NE) at the neurula stage. Their interaction is essential for restricting the TGF-β signal to the left lateral plate mesoderm (LPM). We find that the same posterior region also expresses another TGF-β ligand, derrière. Its expression is bilateral, preceding both Coco and Xnr1, and depletion of derrière protein on the left side leads to absence of Xnr1 expression in the left LPM and a randomized left–right axis. In the NE, derrière depletion reduces Xnr1 expression, and derepresses its own expression throughout the medial paraxial mesoderm. Like Xnr1, derrière interacts directly with Coco, which inhibits its biological activity. We propose of model where derrière and Xnr1 activity are limited to the left side of the NE by asymmetric Coco function.

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The Role of the Pitx2c N-terminus in Left-Right Patterning

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Pitx2c, a member of the bicoid family of homeodomain proteins, is asymmetrically expressed in the left lateral plate mesoderm and in many of the organs that become asymmetrically positioned relative to the midline. Its asymmetric expression on the left side is downstream of Nodal signaling and evolutionarily conserved. Overexpression of full-length Pitx2c on the right side of the embryos randomizes the direction of heart looping and gut situs. Left-sided misexpression of the Pitx2c N-terminus and homeodomain fused to a transcriptional repressor domain antagonizes the normal rightward looping of the heart (Yu et al., 2001). Here, we report that overexpression of the Pitx2c N-terminus alone on the left side of the embryo randomized the direction of heart looping, suggesting that it possesses a dominant negative activity. We performed mutagenesis analysis of the Pitx2c N-terminus and identified a 5 amino acid sequence that is critical for its ability to antagonize normal left-right patterning. We hypothesize that this region participates in protein interactions required for Pitx2c's left-right patterning activity. We have performed protein interaction assays to identify proteins that specifically interact with the wild type Pitx2c N-terminus but not with the mutant. Sequence analysis and functional data for these proteins will be presented.

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Chick Claudin-1 function in the establishment of the left–right axis

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In vertebrates, cascades of signaling molecules on the left and right sides of the embryo are responsible for directing the developmental events that lead to asymmetric organ development and positioning. We have isolated Claudin-1, a component of epithelial tight junctions, in a subtractive screen to identify novel molecules involved in asymmetric morphogenesis. Claudins are responsible for regulating the size and ion-selective permeability of tight junctions. Retroviral overexpression of chick Claudin-1 on the right side of HH stage 4–8 chick embryos randomizes the direction of heart looping. The Claudin-1 cytoplasmic C-terminus contains a protein kinase C (PKC) site at amino acid 206. Since phosphorylation of the cytoplasmic domains of Claudins has been shown to affect tight junction function, we prepared a series of construct where the C-terminus of Claudin-1 was either deleted or T206 was mutated to a neutral residue (alanine), a negatively charged residue (glutamic acid) or a positively charged residue (arginine). Wild type and mutant Claudin-1 proteins co-localize with Z0-1 at the cell membrane of stably transfected HEK293 cells. However, in gain-of-function experiments, these mutants are not able to randomize the direction of heart looping as was seen for wild type